BACTERIAL INSECTICIDE PRODUCTION: POTENTIAL USE OF WASTE SLUDGES FROM PULP AND PAPER INDUSTRIES

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ABSTRACT

At present, one of the most studied and commercially available biocontrol agent of insect pests is the *Bacillus thuringiensis*. Its use is permitted up to harvest time on several crops used for fresh consumption, and also on other crops and, more recently, on stored grains [1].

Bacillus thuringiensis products have attributes that are essential for a sucessfull microbial pesticide: specificity, potency and efficacy against several insects, in addition to the economic viability of production by fermentation process and safety to man, animals and beneficial insects. By fermentation the bacillus produces a proteinaceous cristal along with one toxic active spore and this parasporal body is also a lethal toxin for certain insects.

The media for industrial production of this bacterium are basically composed of complex carbon and nitrogen sources. In Brazil, the production--process by means of submerged culture is patented, using sugar cane molasses and corn steep liquor as nutrient medium [2]. Other agricultural by-products available have not yet been explored as potential components.

Based on the promising results obtained in laboratory and pilot plant production [4], the CNPDA with the cooperation of UNICAMP initiated studies to employ new methods of fermentation to spare the need of large aerated fermentors.

To obtain an economical production, it requires that the fermentation medium has to be as cheap as possible, as well as able to support conveniently the endotoxin production.

The work reported in this paper is part of a study on development of a culture medium based on agro-industrial by-products for production of microbial insecticides, using waste sludger from pulp and paper industries.

INTRODUCTION

Under current agricultural practice, it has been estimated that more than 40% losses of crops in Latin America are due to insect pests, in spite of the heavy and extensive use of chemical pesticides, these losses are occuring annually.

Chemical pesticides, particularly chemical insecticides, are toxic to non target species (including beneficial insects), domestic animals and man and they have been steadily losing effectiveness due to the development of resistence among target species and it is important to replace the chemical pesticides by biorational agents that would be specific in their action.

The toxic activity associated with *B. thuringiensis* takes place primarily in the parasporal crystal formed within the mother cell during sporulation, either "in vivo" or "in vitro". This crystal is composed of a glycoprotein subunit that is believed to be a protoxin which is converted to a toxin by proteolytic activity.

In addition to the crystalline inclusion, some *B. thuringiensis* varieties release a nucleotide-like particle, called beta-exotoxin, during the vegetative growth phase. Other insecticide metabolites produced by some strains of this bacterium - alfa and gama exotoxins - as well as some enzymes are of only secondary importance for insect control.

A mixture of spores are crystals of *B. thuringiensis* are commercially produced and economic marketed in many countries, as specific insecticide. However, in Brazil economic reasons have restricted the wider use of it. As it is produced by fermentation means, fermentation technology in Brazil has to aim the production of these endotoxins at reduced costs. These costs depend on the efficiency at which the organims produces the product (choice. of the proper strain, the fermentation condition, the means by which the product is recovered from the broth, and so on).

Bacillus thuringiensis fermentation process

The practical use of entomopathogenic microorganisms for crop protection, is only possible if a large scale industrial production is developed. This principle was understood even by the early workers who attempted to industrialize their cultivation process for *B. thuringiensis* in the middle of the twentieth century [5], [6]. Dulmage [7], [8], [9], successfuly used agroindustrial by-products in submerged fermentation for producing active delta-endotoxins. In the same line, attempts were made by Foda (1984) apud [10], to employ new methods of fermentation in the hope to arrive at a less expensive and more feasible method for that production. They employed a semi-solid type of fermentation where the bacterium was grown in trays containing thin layers of the semi-solid medium with agar. This technique provide promising results in endotoxin yields, and potential biological activity.

In order to detect the possible use of solid residues and sludges from agroindustrial industries, as culture media, many efforts were done to develop a semi-solid technique.

Semi-solid fermentation means the growth process of microorganisms on solid materials, not in a liquid phase. In this type of fermentation the substrate may be put on a flask and, after inoculation, the microorganism develops on this substrate. The flask can be occasionally shaked to promote the necessary aeration.

In the search for a new substrate for *B. thuringiensis* production, a selection was made based primarily on the following guidelines:

- agroindustrial solid by-products, available in the region where the commercial production was going to be done;
- the by-product must have a stable and nearly constant composition;
- it must be really a by-product or residue, of low cost.

Base on these guidelines, many contacts were made with different industries to get information about their residues, their mean chemical composition, the cost and quantities generated. From these data it was selected some by-products to be used in the experiments, among them, the sludge from pulp and paper industry.

In Brazil there are many pulp and paper industries, using wood trees (*Eucalyptus grandis*), that generate several types and big quantities of residues (liquid and sludges).

The sludge used in this work have the average composition presented in Table 1, where we noted a high content of carbohydrate and nitrogen material, beside the mineral salts present.

TABLE 1

Mean chemical composition of the sludge from pulp and paper industry

Component		Component	
Organic matter	60,66%	Phosphorus	0,09%
Moisture	81,60%	Potassium	0,026%
Total Carbon	35,19%	Calcium	5,25%
Total Nitrogen	0,22%	Manganese	0,09%
Sodium	0,14%		

In this way this sludge was used as culture medium in semi-solid fermentation of *B. thuringiensis* to be used as biological insecticide against lepidopterous pests. Biassay tests were done with *Anticarsia gemmatalis* a soybean insect pest.

During fermentation in the semi-solid process, drying of the culture medium occurs and it was necessary to add aqueous solution in order to adjust the humidity level need for bacterial growth and sporulation.

RESULTS

Based on the results showed on Tabels 2 and 3, it is observed that semi-solid fermentation process can be successfully used for *B. thuringiensis* spore production when the substrate is the sludge from pulp and paper industry. This sludge can be used as a complete medium for the development of *B. thuringiensis* or can be enriched with a mineral salt source.

TABLE 2

Influence of periodic humidifcation on the production of *Bacillus thuringiensis* spores, by semi-solid fermentation process (typical results)

Harvest time (h)	Moisture content (%)			Maximum number of viable spores/g of fermented medium at harvest time		
	Water added / flask			Water added / flask		
	0 ml	1 ml	2 ml	0 ml	1 ml	2 ml
0	50.17	50.17	50.17	4.5×10^{4}	4.5×10^{4}	4.5×10^{4}
96	47.50	50.85	51.40	9.9×10^{10}	8.9×10^{14}	9.2×10^{12}
198	.43.16	52.90	57.71	10^{10}	1.1×10^{17}	1.0×10^{23}
288	40.49	53.36	59.98	1.4×10^{9}	5.3×10^{13}	1.0×10^{32}
336	38.00	54.15	62.10	1.2×10^{9}	1.8×10^{12}	4.0×10^{32}

TABLE 3

Bioassay of the fermented product against *Anticarsia gemmatalis*

Bt spores / ml applied on the soybean leaf	% Mortality
1.9×10^{9}	100
1.9×10^{6}	80
2.3×10^{10} (*)	100
Control	24

(*) As standard it was used the commercial product DIPEL produced by Abbott Laboratories.

CONCLUSIONS

Due to its safety and specificity, the microbial insecticides, as biocontrol agents, have found wide acceptance particularly those produced by B. *thuringiensis*.

The universality of the sludge used in this work, from pulp and paper industry, and the possible use of semi-solid fermentation process with such a low price substrate enable us to continue this research searching for techniques of recovering the insecticide and standardizing it, to use against the lepidoptera pests, that in Brazil, are in a big quantity and diversifity, causing around 40% losses on several crops and stored grains.

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